PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark
Office, PCT
2011 South Clark Place Room
CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 05 April 2001 (05.04.01)	
International application No. PCT/GB00/02743	**Applicant's or agent's file reference** SMW/BP5868229
International filing date (day/month/year) 17 July 2000 (17.07.00)	**Priority date** (day/month/year) 16 July 1999 (16.07.99)
Applicant DE LA CUEVA MENDEZ, Guillermo et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

13 February 2001 (13.02.01)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Anman QIU
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

WALTON, Seán, M.
Mewburn Ellis
York House
23 Kingsway
London WC2B 6HP
ROYAUME-UNI

Date of mailing (day/month/year)
22 February 2001 (22.02.01)

Applicant's or agent's file reference
SMW/BP5868229

IMPORTANT NOTIFICATION

International application No.
PCT/GB00/02743

International filing date (day/month/year)
17 July 2000 (17.07.00)

1. The following indications appeared on record concerning:

[X] the applicant [ ] the inventor [ ] the agent [ ] the common representative

Name and Address
CANCER RESEARCH CAMPAIGN
TECHNOLOGY LIMITED
Cambridge House
6-10 Cambridge Terrace
Regent's Park
London NW1 4JL
United Kingdom

State of Nationality: GB
State of Residence: GB
Telephone No.:
Facsimile No.:
Teleprinter No.:

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[X] the person [X] the name [ ] the address [ ] the nationality [ ] the residence

Name and Address
CANCER RESEARCH VENTURES LIMITED
Cambridge House
6-10 Cambridge Terrace
Regent's Park
London NW1 4JL
United Kingdom

State of Nationality: GB
State of Residence: GB
Telephone No.:
Facsimile No.:
Teleprinter No.:

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[ ] the International Preliminary Examining Authority
[X] the designated Offices concerned
[ ] the elected Offices concerned
[ ] other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Facsimile No.: (41-22) 740.14.35

Authorized officer
S. Buttay
Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION**	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
SMW/BP5868229		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/GB 00/ 02743	17/07/2000	16/07/1999
Applicant		
CANCER RESEARCH CAMPAIGN TECHNOLOGY LIMITED et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [X] furnished subsequently to this Authority in written form.

   [X] furnished subsequently to this Authority in computer readble form.

   [X] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [X] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [ ] the text is approved as submitted by the applicant.

   [X] the text has been established by this Authority to read as follows:

   METHODS EMPLOYING BACTERIAL TOXIN-ANTITOXIN SYSTEMS FOR KILLING EUKARYOTIC CELLS

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [X] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
[redacted]/GB 00/02743

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K38/16 A61K39/02 A61K48/00 A61K45/06 A61K31/713
A61P35/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, BIOSIS, CANCERLIT, CHEM ABS Data, MEDLINE, SCISEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HAMBLETON ET AL.: "Antitoxins and botulinum toxin treatment" BRITISH MEDICAL JOURNAL, vol. 304, 1992, pages 959-960, XP000944661 * see introduction and page 960 left col.* ---	18
X	WO 94 05345 A (KABAKOV VIKTOR GRIGORIEVICH ;SELEZOV EUGENY AFANASIEVICH (RU); SKO) 17 March 1994 (1994-03-17) * see pages 26-27, 32-36 and claims 7-13 * ---	1,4,6,10
A	M. HOLCIK ET AL.: "Conditionally lethal genes associated with bacterial plasmids." MICROBIOLOGY, vol. 143, 1997, pages 3403-3416, XP000941408 * see abstract * --- -/--	1-19

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
7 December 2000	28/12/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Gore, V

2

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
[redacted]/GB 00/02743

C.(Continuation) DOCUMENTS CONSID[redacted]D TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	MAGNUSON R. ET AL.: "Corepression of the P1 operon by Phd and Doc." J. BACTERIOL., vol. 180, no. 23, 1998, pages 6342-6351, XP000942967 * see abstract and page 6350 *	1-19
A	RAWLINGS D.E.: "Proteic toxin-antitoxin, bacterial plasmid addiction systems and their evolution with special reference to the ps system of pTF-FC2." FEMS MICROBIOL. LETTERS, vol. 176, 15 July 1999 (1999-07-15), pages 269-277, XP000942964 * see abstract *	1-19
X,P	WO 99 58652 A (KRISTOFFERSEN PETER ;GERDES KENN (DK); GROENLUND HUGO (DK); PEDERS) 18 November 1999 (1999-11-18) * see abstract, pages 3-4, page 8 lines 14-17, claims 51, 60, 79 and 87-88 *	1,2,4-6, 10-13,18

2

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
[redacted]T/GB 00/02743

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 9405345	A	17-03-1994	CA	2122600 A,C	17-03-1994
			CH	685675 A	15-09-1995
			DE	4295020 C	31-07-1997
			DE	4295020 T	20-10-1994
			FR	2716940 A	08-09-1995
			JP	7500765 T	26-01-1995
			SE	511883 C	13-12-1999
			SE	9401476 A	29-04-1994
			US	5586872 A	24-12-1996
WO 9958652	A	18-11-1999	AU	3596399 A	29-11-1999

14

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
SMW/BP5868229		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB00/02743	17/07/2000	16/07/1999

International Patent Classification (IPC) or national classification and IPC
A61K38/16

Applicant
CANCER RESEARCH CAMPAIGN TECHNOLOGY LIMITED et al.

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☒ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
13/02/2001	10.10.2001

Name and mailing address of the international preliminary examining authority:

European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
Fax: +49 89 2399 - 4465

Authorized officer

Merckling, V

Telephone No. +49 89 2399 8590

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB00/02743

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-50 as originally filed

Claims, No.:

1-19 as originally filed

Drawings, sheets:

1-6 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 1,3-17.

because:

☒ the said international application, or the said claims Nos. 1,3-17 relate to the following subject matter which does not require an international preliminary examination (*specify*):
see separate sheet

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos. .

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N) Yes: Claims 1-19

	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-19
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	2,18-19 (YES), 1,3-17 see separate sheet
	No:	Claims	

2. Citations and explanations
see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

1. Reference is made to the following documents :

   D1 : Hambleton et al. (1992)
   D2 : WO-A-9426308
   D3 : Holcik et al. (1997)
   D4 : Magnuson et al. (1998)
   D5 : Rawlings (15.07.99)
   D6 : WO-A-9958652

Regarding point III

2. Claims 1 and 3-17 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

Regarding point V

3. For the assessment of the present claims 1 and 3-17 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

4. In D1, patients treated with botulinum toxin (BT) for torticollis and showing decreasing responses to BT are tested for anti-BT immune response. In order to detect the presence of anti-BT antibodies, serum samples are assayed by *in vivo* toxin neutralization tests (see introduction and page 960 left col.).
D2 discloses the combined use of toxins and antitoxins in therapy for preventing endogenous production of antibodies to the toxin or other unwanted side-effects (see abstract). Preferred toxins are bacteriotoxins, especially from Clostridium (see page 26 lines 5-13 and claims 7-13). Antitoxin is preferably an anti-toxin antibody or fragment thereof (cl.15). In the case of local administration to tumors, the toxin and the antitoxin are preferably administered concomitantly (pages 26-27). The combination of toxin and antitoxin may be used for treating solid tumors (see page 32). However, no single composition comprising a toxin and the corresponding antitoxin is disclosed. The two compounds are always administered in two different pharmaceutical compositions, and not necessarily at the same site (see pages 32-36).
D3 is a review article on conditionally lethal genes associated with bacterial plasmids and on post-segregational killing systems in general (see abstract). Most of the systems cited in the present application are described, for example ccd, kis/kid (pages 3404-3406), HigA and HigB, hok and sok (page 3407), kic, kil and kor (page 3410). It is also mentioned that the killer protein of parD is likely to be an inhibitor of DNA-B dependent DNA replication (page 3406 left col.). But the use of such systems in therapy or in eukaryotic cells is not described.
D4 describes another toxin/antidote system encoded by plasmid P1, the PhD-Doc system. This system is compared to other known toxin-antidote systems, but only in *E.coli* (see abstract and page 6350). There is no suggestion of medical use or transfection in eukaryotic cells.
D5 is also a review on post-segregational killing systems in bacteria, with no reference to eukaryotic cells.

4.1 D1 explicitly discloses a composition comprising both a toxin (BT in that case) and a toxin inhibitor (anti-BT antibodies).However, this composition is then injected into mice, in order to measure the level of ant-BT antibodies contained in the patients'

serum. This cannot be regarded as a therapeutic or diagnosis use Consequently, claims 18-19, that are directed to a first medical use, are novel.

4.2 Claims 1, directed to an *in vivo* or *in vitro* method for inhibiting proliferation of eukaryotic cells comprising administering within a eukaryotic cell a bacterial toxin and a toxin inhibitor (or nucleic acids encoding both proteins). As a matter of fact, D2 discloses the combined use of a toxin and an inhibitor of said toxin for treating tumors. In the definition of the present application, the general expressions "selective cell cycle inhibition" and "killing target cells" covers the use for treating tumors (see present claim 16). It should nevertheless be stressed that D2 does not teach to provide both the toxin and the antitoxin within a eukaryotic cell. The toxin is a small molecule that is easily taken up by cells, even when administered systemically. The core of the invention, in D2, resides in the neutralization of excess toxin molecules that have not been taken up by cells. The antitoxin that are used for neutralization are antibodies that are injected systemically and that do not enter the cells (see pages 26-27 and page 34). It follows that D2 does not teach the administration of a toxin and an antitoxin within a eukaryotic cell. A composition comprising a nucleic acid encoding a toxin, and antitoxin, has not been disclosed either. Claims 1-10 are new.

4.3 None of the available documents discloses the use of a toxin of a post-segregational killing system for killing eukaryotic cells. The subject-matter of claims 11-17 is new.

5. D2, considered as the closest prior art, discloses the use of a toxin and a toxin inhibitor, in two separate compositions but administered concomitantly, for treating tumors. The problem solved by D2 is to minimize the side effects due to the toxin and/or avoid the occurrence of immune responses directed against the toxin. Since these problems are only relevant in the case of *in vivo* administration, the use of combined toxin and antitoxin for killing cells *in vitro* cannot be derived from D2 in an obvious manner. Claims
The use of a toxin/antitoxin system in plant is not suggested either in any of the available prior art documents. Claims 1-10 are inventive.

5.1 D2 does not mention any bacterial toxin of a post-segregational killing system. The available documents dealing with this type of bacterial toxins do not suggest that

these toxin could have an effect on eukaryotic cells. Consequently, claims 11-17 are inventive.

5.2 As for claims 18-19, D1 does not point to any possible medical use of a composition comprising a toxin and an antitoxin. In D2, the toxin and the antitoxin are never mixed in a single composition. The aim of the use of an antitoxin in addition of a toxin is to bind the excess toxin (i.e. toxin not bound to the tumor) in order to avoid damage to normal tissue and endogenous immune response (see page 34). Regarding this, it would not be logical to combine the toxin and the antitoxin in a single composition because the toxin might be neutralized to a large extent even before reaching its target. The person skilled in the art confronted to the problem of killing target cells would not be prompted by D2 to use a composition according to claim 18. Claims 18-19 are not obvious.

Regarding point VI

6. Document D6 is not regarded as part of the prior art during the International Examination Phase. However, it could be taken into account for the assessment of novelty in Regional Phase and would appear to be very relevant.